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cDNA by Tth-like DNA polymerase activity. The final double-stranded cDNAs are preferably cloned into competent vectors for further applications, such as transfection assay, differential screening, functional detection and so on.

2. In page 8, line 6 to 20, please delete the second paragraph and replace such deleted paragraph with the following replacement paragraph which is the same as the original filed paragraph:

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The present invention is directed to a novel polymerase chain reaction method for mRNA amplification from single cells, named "RNA-polymerase chain reaction (RNA-PCR)". This method is primarily designed for differential screening of tissue-specific gene expressions in cell level, cloning full-length sequences of unknown gene transcripts, generating pure probes for hybridization assays, synthesizing peptides in vitro, and preparing complete cDNA libraries for gene chip technology. The purpose of the RNA-PCR relies on the repeating steps of reverse transcription, denaturation, double-stranded cDNA synthesis and in vitro transcription to bring up the population of mRNAs to two thousand folds in one cycle of above procedure. In brief, the preferred version (FIG.1) of the present invention is based on: 1) prevention of mRNA degradation, 2) first reverse transcription and terminal transferase reaction to incorporate 3'-polynucleotide tails to the first-strand cDNAs, 3) denaturation and then double-stranded cDNA formation based on the extension of specific promoter-primers complementary to the 3'-polynucleotide tails, 4) transcription from promoter to amplify mRNAs up to two thousand folds per round, and 5) repeating aforementioned steps to achieve desired RNA amplification.

3. Please delete the paragraph between page 8, line 21 and page 9, line 12, and replace such deleted paragraph with the following replacement paragraph which is the same as the original filed paragraph:

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Alternatively, the second preferred version (FIG.2) of the present invention is based on: 1) prevention of mRNA degradation, 2) first reverse transcription to incorporate first promoters to the 5'-ends of first-strand cDNAs and then addition of polynucleotide sequences to the 3'-ends of the first-strand cDNAs, 3) double-stranded cDNA synthesis based on the extension of second promoter sequences complementary to the 3'-polynucleotide regions of the first-strand cDNAs, 4) transcription to amplify either aRNAs or mRNAs up to two thousand folds in the first round of amplification cycle, and 5) repeating aforementioned cycling steps to achieve desired amount of RNAs. As shown in FIG.2, the first promoter used here is different from the second promoter, resulting the control of transcription by adding different RNA polymerases. The first promoter is incorporated for aRNA amplification, whereas the second promoter is designed for mRNA amplification. By this way in conjunction with a reverse transcription step, we can choose to amplify aRNAs, first-strand cDNAs, mRNAs or second-strand cDNAs of interest, depending on which RNA polymerase and nuclease we use. Although the second and third preferred embodiments (FIGS.2 and 3) are more complicated than the first preferred embodiment (FIG.1), the principle and broad features of the second and third preferred embodiments are completely within the scope of the first preferred embodiment of the present invention.

4. Please delete the last paragraph in page 9, line 13 to 27, and replace such deleted paragraph with the following replacement paragraph which is the same as the original filed paragraph:

C4
As used herein, the first-strand complementary DNA (cDNA) refers to a DNA sequence which is complementary to a natural messenger RNA sequence in an A-T and C-G composition. The antisense RNA (aRNA) refers to an RNA sequence which is complementary to a natural messenger RNA sequence in an A-U and C-G composition. And, the oligo(dT)-

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promoter sequence refers to an RNA polymerase promoter sequence coupled with a poly-deoxythymidylate (dT) sequence in its 3'-end, of which the minimal number of linked dT is seven; most preferably, the number is about twenty-six. The sense sequence refers to a nucleotide sequence which is in the same sequence order and composition as its homolog mRNA, whereas the antisense sequence refers to a nucleotide sequence which is complementary to its respective mRNA homologue. On the other hand, the oligo(antisense polynucleotide)-promoter sequence refers to an oligonucleotide sequence which is complementary to the polynucleotide-tail of said polynucleotide-tailed cDNAs and also linked to an RNA polymerase promoter in its 5'-end. And, Tth-like DNA polymerases refer to RNA- and DNA-dependent DNA polymerases with reverse transcription activity.

5. Please delete the first paragraph in page 10, line 1 to 16, and replace such deleted paragraph with the following replacement paragraph which is the same as the original filed paragraph:

C5
By improving the methods of in-vitro transcription and in-cell RT-PCR (Embleton et al., *Nucleic Acid Res.* (1992)), we invent the cycling amplification of intracellular full-length mRNAs. This cycling procedure preferably starts from reverse transcription of intracellular mRNAs with Tth-like DNA polymerase, following a tailing reaction with terminal transferases and then denaturation of resulting mRNA-cDNA hybrid duplexes. After renaturation of above tailed cDNAs to specific promoter-linked primers, double-stranded cDNAs are formed by Tth-like DNA polymerases. And then, promoter-specific RNA polymerase(s) is added to accomplish the transcriptional amplification of intracellular mRNAs. The novelties of this amplification cycling procedure of the present invention are as follows: 1) single copy rare mRNAs can be increased up to 2000 folds in one round of amplification without mis-reading mistakes, 2) the mRNA amplification is linear and does not result in preferential amplification of abundant mRNA species, 3) the mRNA degradation is inhibited by fixation, and 4) the final mRNA products are of full-length and can be directly used to generate a complete cDNA library or synthesize proteins in vitro (Shi-Lung Lin et al. *Nucleic Acid Res.* (1999)).

IN THE CLAIMS:

Please rewrite claims 1, 8-10, 12, 16-17, 30, and 33 of record as follows:

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Claim 1 (amended). A method of generating amplified messenger RNAs with polymerase reaction activity, comprising the steps of:

- (a) providing a plurality of intracellular messenger RNAs for following steps
(b) to (f);
- sub E